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Review Article—

The High Susceptibility of Turkeys to Influenza Viruses of Different Origins Implies Their Importance as Potential Intermediate Hosts

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SUMMARY. Several previous reports and our studies show that waterfowl-origin influenza viruses can be more easily transmitted to domestic turkeys than chickens. Similarly, studies indicate turkeys to be better hosts for low pathogenic avian influenza viruses isolated from commercial poultry operations and live bird markets in comparison to chickens. Low 50% infectious-dose titers of wild bird as well as poultry-adapted viruses for turkeys further suggest that turkeys can be easily infected following a low-dose exposure. Also, interspecies transmission of swine influenza viruses to turkeys occurs frequently. These findings suggest the role of turkeys as suitable intermediate hosts that can be easily infected with influenza viruses of different origins and that turkeys can act as source of infection for other land-based poultry or even mammals.

RESUMEN. *Estudio Recapitulativo*—La alta susceptibilidad de los pavos al virus de la influenza de diferentes orígenes implica su importancia como posibles huéspedes intermediarios.

Varios reportes previos y los estudios de nuestro laboratorio muestran que los virus de la influenza originados de aves acuáticas puede ser más fácilmente transmitidos a los pavos domésticos que a los pollos. De la misma manera, los estudios indican que los pavos son mejores hospederos que los pollos para virus de la influenza aviar de baja patogenicidad aislados de operaciones avícolas comerciales y de mercados de aves vivas. Títulos bajos de dosis infecciosas 50% de virus con origen en aves silvestres así como virus adaptados en pavos, sugieren que los pavos pueden ser fácilmente infectados después de una exposición con dosis virales bajas. Además, ocurre con frecuencia la transmisión entre especies por el virus de la influenza porcina a los pavos. Estos hallazgos sugieren el papel de los pavos como huéspedes intermediarios que pueden ser fácilmente infectados por virus de la influenza de diferentes orígenes y que los pavos pueden actuar como fuente de infección para otras especies aviares comerciales, o incluso mamíferos.

Key words: turkeys, influenza, interspecies transmission

Abbreviations: EID_{50} = egg infectious dose; HI = hemagglutination inhibition; Gal = galactose; LBM = live bird market; LPAI = low pathogenic avian influenza; MAA = *Maackia amurensis* agglutinin; RRT-PCR = reverse transcriptase–polymerase chain reaction; SA = sialic acid; SNA = *Sambucus nigra* agglutinin; TR = triple-reassortant

Wild waterfowl, gulls, and shorebirds are believed to be the natural hosts and reservoirs of influenza A viruses (53). Influenza viruses in all other species of mammals and birds are derived from the wild bird reservoirs, in which all the 16 hemagglutinin and 9 neuraminidase subtypes have been found (11,60). In these reservoirs, influenza viruses are considered to be in a state of evolutionary stasis and infections are usually asymptomatic. Though they are not natural hosts, different species of domestic poultry have been found to be infected with influenza viruses as a result of exposure to wild bird reservoirs or contaminated environment from these reservoirs. In most cases, low pathogenic influenza infections in domestic poultry result in mild respiratory disease, reduction in egg production, and occasionally increased mortality (9). Surveillance studies indicate that low pathogenic influenza infections are widespread among commercial poultry. Among domestic bird species, infections have been most commonly reported from Galliformes and Anseriformes, which include chickens, turkeys, quails, guineafowl, pheasants, patridges, ducks, and geese. Psittaciformes (parrots, cockatoos, parakeets), Casuariiformes (emu), Struthioniformes (ostrich), and Rheiformes (rhea) have been found to be susceptible under natural conditions. Infections of some free-living birds like starlings and sparrows have been found following their close association with infected birds in poultry farms (9,53). Experimental infections also indicate the susceptibility of different domestic bird species, including chickens, ducks, turkeys, quails, pigeons, and pheasants to influenza viral infections from different sources (23,27,32,37,38,45,46,48,49). In addition to lowered production, increased mortality, and accompanying economic losses, major concerns with infection of poultry with low pathogenic influenza viruses are the ability of these species to act as bridging hosts for influenza infections from wild bird reservoirs to other land-based poultry and mammals including humans. Also, there is threat of these viruses mutating to the highly pathogenic forms after many replication cycles in poultry (37,60). Among the different domestic birds that can be infected with influenza viruses, chickens and turkeys have gained importance in the United States, considering their economic and public health significance.

Turkeys are susceptible to many wild aquatic bird influenza viruses. It has been documented that multiple subtypes of influenza viruses can establish stable lineages in domestic poultry (29,50,59). Though the source of influenza infections for domestic bird species are believed to be wild bird reservoirs, studies indicate that domestic

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poultry can be infected with influenza viruses from different sources, for example, swine influenza infections of turkeys. Avian influenza infections have been recorded from turkeys worldwide (1,2,3,7,33,55). Similarly, multiple subtypes of influenza viruses have been isolated from healthy and diseased chickens; however, it is not known whether the influenza virus was transmitted directly to chickens from an aquatic bird, or if the virus acquired expanded host-range capabilities by replication in other avian species, prior to infection of chickens. Several observations show that waterfowl origin influenza virus can be more easily transmitted to domestic turkeys than chickens. In the United States, since the mid-1960s, influenza virus infections have been seen consistently in turkeys in California and Minnesota, where turkeys were range reared and farms were heavily concentrated and situated on migratory waterfowl flyways (20). Campitelli *et al.* identified for the first time that the H7N3 viruses from turkeys in Italy were derived directly from influenza strains circulating in wild waterfowl (7). Several previous studies and field data document that turkeys may be more susceptible to wild bird influenza viruses than chickens (19,48,54). Studies on replication and intraspecies transmission characteristics of eight low pathogenic H5 subtype wild bird isolates (one from mute swan, four from mallards, and four from ruddy turnstones) in chickens and turkeys in our lab also indicated the higher susceptibility of turkeys to mallard and ruddy turnstone viruses in comparison to chickens (unpublished data).

Studies on the minimum infectious dose of wild bird viruses for different domestic bird species are limited. Determination of 50% infectious dose titers of wild bird viruses of H5 subtype indicated >1000 times higher dose for infection of chickens in comparison to turkeys (48,54, unpubl. data). The mean 50% infectious dose titers for different wild bird isolates for chickens were in the range of $10^{6.3}$–$10^{8.3}$ 50% egg infectious dose (EID_{50}), whereas for turkeys, the range was from $10^{2.5}$ to $10^{4.2}$ EID_{50}. Although data from other subtypes are not available, the low minimum infectious dose suggests that turkeys can be easily infected following a low dose exposure and confirms the higher susceptibility of turkeys to influenza viruses of wild bird origin, especially of the H5 subtype. Their high susceptibility also indicates that turkeys could be good sentinel hosts in influenza surveillance studies and/or better model hosts for studies on adaptation of wild bird origin influenza viruses for domestic poultry. Once domestic birds are infected, influenza viruses in them undergo rapid evolution (17,66). It is possible that wild bird viruses circulating in turkeys could undergo favorable changes that permit their adaptation to chickens and other domestic poultry. Hence, the potential role of turkeys as bridging hosts for introduction of low pathogenic waterfowl-origin influenza viruses into other domestic poultry cannot be ruled out. Although confinement rearing has become the norm in the United States and other developed countries, turkeys could be significant players in supporting and transmitting influenza infections to other land-based poultry where wild birds have free access to domestic poultry.

Influenza viruses isolated from domestic birds can replicate and transmit among turkeys. Though detailed experimental studies on the replication and transmission characteristics of domestic bird-origin influenza viruses in turkeys are lacking, available evidence indicates susceptibility of turkeys to low pathogenic avian influenza (LPAI) viruses isolated from commercial poultry operations and live bird markets (26,54,56). Among commercial poultry operations, more LPAI outbreaks have been reported in turkeys in comparison to chickens (53). Experimental studies with a LPAI H7N2 isolate that caused an outbreak among commercial poultry primarily infecting turkeys indicated a lower 50% infectious dose titer for turkeys than chickens. Also, 20–158-fold higher infectious virus was recovered from turkeys in comparison to chickens (56). A similar outbreak with H7 subtype viruses in poultry in Italy in 1999 primarily affected turkey flocks (64). The high susceptibility of turkeys might be explained by the low minimum infectious dose (about 10-fold less viral load for infection of turkeys in comparison to chickens) required for infection. Our studies using a highly poultry-adapted strain of LPAI, A/parrot/California/04, showed a lower 50% infectious dose titer of $10^{1.4}$ 50% EID_{50} /0.2 ml for turkeys in comparison to a $10^{2.6}$ EID_{50}/0.2-ml dose for chickens. However, it should be noted that minimum infectious dose required may vary with virus strains as well as the genetic constitution of the birds used in the studies. Hence, caution should be undertaken when generalizing the results from studies using few isolates on a particular turkey breed. Also, under field conditions, where secondary infections or other factors can reduce immune responses, susceptibility and clinical disease may be exacerbated. The higher susceptibility in an outbreak area may also be an indication of the higher population of turkey flocks in the outbreak area. In spite of the limitations of experimental infection studies, they are important to understand the biological properties of viruses and host ecologies. In addition to wild bird isolates as previously described, 12 low pathogenic influenza virus isolates (which includes duck, chicken, pheasant, and emu isolates) from live bird markets (LBMs) as well as chicken and turkey isolates from commercial poultry operations also revealed turkeys to be highly susceptible hosts for influenza viruses of different degrees of adaptation for domestic poultry (unpubl. data). These viruses were inoculated at a dose of $10^{6.0}$ EID_{50}/0.2 ml through choanal route to chickens and turkeys. Influenza isolates from LBMs are believed to be highly poultry adapted, as they provide favorable environment for adaptation of influenza viruses for a variety of hosts, including chickens, turkeys, pheasants, quails, and ducks, and have been implicated as the source of influenza outbreaks in commercial poultry operations as well as in humans. LBMs in New York and New Jersey have been implicated as significant sources of influenza viruses for commercial chicken and turkey operations in the United States (36,42). The high susceptibility of turkeys to domestic bird isolates again underscores their importance as potential bridging hosts for influenza viruses from different origins and domestic poultry.

Interspecies transmission of swine influenza viruses to turkeys: a frequent event. Currently three subtypes of influenza viruses are commonly found in pigs worldwide, H1N1, H1N2, and H3N2 (5,40). These viruses were found to be derived either from mammalian or avian viruses or their reassortants (6,8). H1N1 influenza viruses were first isolated from pigs in the United States in 1930 (43), and until the late 1990s, classical H1N1 lineage was the predominant influenza subtype in pigs in the United States (34). The first reported isolation of swine influenza viruses in turkeys was in 1980–81 (22). Since then, transmission of swine H1N1 influenza viruses to turkeys has been documented several times (4,10,28,30,61) and in the majority of the cases, the turkey flocks were housed in close proximity to swine herds (51). In 1998, triple-reassortant (TR) influenza viruses containing gene segments derived from recent human (HA, NA, and PB1), swine (NS, NP, and M), and avian (PB2, PA) viruses were first isolated from pigs and since then, have spread over much of the U.S. swine population (58,65). Since 2003, the swine-origin TR H3N2 viruses have been isolated from the U.S. turkey population (8,55). The early reports of swine TR H3N2 viruses crossing the species barrier to infect turkeys were from farms in Minnesota and North Carolina in 2003 (8). Later, the TR H3N2 viruses have been isolated from turkey flocks in different

parts of the United States (55,63). In 2002, Suarez *et al.* reported the isolation of TR H1N2 influenza virus with gene segments derived from swine, human, and avian lineages from turkey breeder hens associated with sudden drops in egg production (51). This virus was also believed to have a swine source of infection and provides additional evidence of cross-species transmission of swine influenza viruses to turkeys. Among the different domestic bird species that are susceptible to swine influenza viruses, including chickens, more field cases have been reported with turkeys, indicating that turkeys may be naturally more susceptible than chickens and other domestic poultry (41,42). Experimental infections also supported the findings that turkeys are more susceptible to influenza infections than chickens. The ability of the turkey TRs to replicate in quail, but not the swine TRs, also indicate the potential role of turkeys as bridging hosts for influenza viruses to other land-based poultry, mammals, or even humans (8). The ability of these swine-origin viruses to infect turkeys is not limited to birds of the younger age group that are usually used for experimental infection studies. Several previous reports document drops in egg production in turkeys due to H1N1 (30,44), reassortant H1N2 (51), and H3N2 viruses (8,35,39,55). Hence, along with the concern of introducing and establishing new influenza lineages in turkeys, interspecies transmission of swine influenza viruses to turkeys can be economically significant. The strong staining for avian receptors and high viral titers in the oviduct and drastic declines in egg production following infection are indicators that influenza viral infections alone without any concurrent infections can result in economic losses under field conditions (39). A recent study reported that turkey embryos are susceptible to swine TR H3N2 influenza viruses, and continuous passage of the swine viruses have been found to result in mutations in HA similar to those found in turkey TR H3N2 viruses (47). However, although embryonated turkey eggs may serve as inexpensive tools to study the adaptation and interspecies transmission of swine influenza viruses, turkey embryos may not mimic the biology of a bird, especially in terms of immune responses and several issues including the reproducibility of the HA mutations with other swine viruses, and the changes in other gene segments should be considered in the study.

The receptor profile in turkeys supports their role as intermediate hosts. Different sialic acid types and linkages in different species of birds and mammals are believed to be barriers for efficient interspecies transmission of influenza viruses. Influenza virus receptors on host cells are believed to be terminal sialic acid (SA) residues that are bound to glycans through an α2,3 or α2,6 linkage, mediated by sialyltransferases that are expressed in a cell- and species-specific manner (12). It is believed that duck intestinal epithelial cells express α2,3SA-galactose (gal) receptors, whereas tracheal epithelial cells of humans mainly express α2,6SA-gal receptors. Avian viruses are believed to bind α2,3SA-gal linked receptors preferentially, and influenza viruses from humans preferentially recognize human-type receptors (31). Apart from the SA-gal linkages to penultimate sugars, the binding of viruses can be affected by structure of more distant parts of the oligosaccharide chain (13,21,52).

Plant lectins, *Maackia amurensis* agglutinin (MAA) and *Sambucus nigra* agglutinin (SNA), that are specific to α2,3SA-gal and α2,6SA-gal terminated sugars are usually used to identify the type of receptors present in different tissue sections. Epithelial cells from trachea and intestines of chickens and quail have been found to have moieties that can bind SNA and MAA and to bind viruses showing human- and avian-type receptor specificity (15,18,57). Our studies using MAA and SNA on turkey tracheal epithelium indicated that turkeys also possess α2,3SA-gal– and α2,6SA-gal–terminated sialyloligosaccharide residues with 80%–90% and 70% of the tracheal epithelial cells, respectively, showing positive staining. The colon sections showed only the presence of α2,3SA-gal receptors (unpubl. data). Previous lectin studies have demonstrated that turkey ovaries contain both α2,3SA-gal and α2,6SA-gal receptors and support our results on the presence of both types of receptors on turkey tissues (25). The presence of avian- and human-type receptors in turkeys explains their higher susceptibility to wild and domestic bird origin and swine viruses. Although different viral and host factors may play roles in successful viral replication, adaptation, and transmission, these findings strengthen the argument that turkeys can be infected with influenza viruses containing mammalian hemagglutinin gene segments and can act as potential intermediate hosts for interspecies transmission and spread of reassortant viruses between birds and humans.

The actual viral binding to the receptors observed have been found to be affected by different factors other than receptor specificity. Avian influenza viruses have been found to bind more strongly to Neu5Ac receptors than to Neu5Gc-containing receptors (21,24,31). Also, duck viruses have been found to bind with greater affinity to Neu5Ac(α2-3)Gal(β1-3)GalNAc-containing receptors than Neu5Ac(α2-3)Gal(β1-4)GlcNAc-containing receptors, indicating that distant parts of the oligosaccharide chains affect binding of the duck viruses (14). Viruses from gulls and shore birds preferentially bind to Neu2Acα2-3gal receptors similar to ducks (16,62). However, H13 viruses were found to bind to 2-3 sialic acid linkages weakly and not to discriminate between β1-3 and β1-4 linkages (16). The length of the gangliosides was also found to have an effect on viral binding. Duck viruses bound to gangliosides with short sugar chains that were found to be abundant in duck intestines, whereas chicken viruses bound more strongly to gangliosides with long sugar chains that were abundant in chicken intestinal tissues (15). As expected, in accordance with the detection of SAα2,3-gal and SAα2,6-gal receptors, epithelial cells from quail and chicken intestines were found to bind both avian- and human-type viruses (18). Similar studies on epithelial cells from trachea and intestines of turkeys are necessary to confirm that they play important roles as intermediate hosts where influenza viruses of avian and human origin can be amplified and spread to susceptible hosts.

CONCLUDING REMARKS

The inherent susceptibility of turkeys to wild bird, domestic bird, and swine origin low-pathogenic influenza viruses signify the important role of turkeys as the domestic bird species that could act as the potential entry points and the bridging species for influenza viral entry into agricultural and commercial poultry systems. Also, the fact that turkeys are easily infected with aquatic bird and swine viruses and the ability of these viruses to reassort increases the probability that further reassortments and evolution of these viruses can take place in these turkey hosts.

Mixing of poultry and outdoor rearing could favor adaptation of low-pathogenic influenza viruses from different sources for domestic poultry and pose serious health risks for birds and other mammals. In the United States alone, turkey consumption has increased more than 100% since 1970, and turkey meat is one of most popular protein choices for consumers. Thus, it is important to control and prevent influenza infection in turkeys, not only to prevent transmission, but also to control the burden of disease in turkeys, to maintain wholesome poultry and poultry product markets.

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